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HDL CHOLESTEROL -PRECIPITANT

Diagnostic reagent for the in-vitro quantitative determination of HDL Cholesterol in human serum and plasma.

REF:BS.1/HC01.050.0050	100 test	REF:BS.1/HC01.100.0100	200 test

CLINICAL SIGNIFICANCE

High density lipoprotein measurement, in conjunction with other lipid determination, has been shown to be useful in assessing the risk of coronary heart disease. HDL is responsible for carrying cholesterol back from preipheral cells to the liver, therefore the risk of coronary heart disease is lowered with increased levels of HDL. usually, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are selectivety precipitated from serum or plasma samples followed by determination of cholesterol in the HDL-containing supernatant.

METHOD PRINCIPLE

low density lipoproteins (LDL) and very low density lipoproteins (VLDL) in sample precipitate with phosphotungstate and magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + + 1\backslash 2\ O2 + H2O \xrightarrow{\text{Chol.oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\ H_2O + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

REAGENT COMPOSITION

R: Reagent	
- Phosphotungstate	- 0.52 mmol/L
- Magnesium chloride	- 30 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the HDL-Cholesterol reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Bioscien HDL-Cholesterol reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored at 15–25˚C.

Deterioration
Do not use The HDL cholesterol reagents if precipitate forms.

SPECIMEN COLLECTION AND PRESERVATION

Serum or plasma

EDTA and Heparin may be used as anticoagulants.
stable for 7 days at 2 – 8 °C, and 4 days at 20-25°C.

SYSTEM PARAMETERS

Wavelength	546 nm (500 – 550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:2.5 0.5 ml 0.2 µl
Temperature	20– 25˚C or 37 ˚C
Incubation time	10 min. at 20–25˚C or 5 min. at 37˚C
Zero adjustment	Reagent Blank
Sensitivity	1mg/dl
Linearity	150 mg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

1-Precipitation

Pipette into centrifuge tubes:

Reagent	0.5 ml
Specimen	0.2 ml

2-Cholesterol- Liquizyme

	Blank	*Specimen*
Distilled water	50 µl	
Specimen supernatant		50 µl
Cholesterol Reagent	1.0 ml	1.0 ml

Mix and incubate for 5 minutes at 37°C or 10 minutes at 20-25°C. Measure absorbance of specimen “A” against reagent blank within 60 minutes.

CALCULATION

HDL-Cholesterol concentration (mg/dl) = (A specimen) × 570

To calculate LDL cholesterol

in mg/dl
LDL Cholesterol = Total cholesterol – HDL Cholesterol – Triglycerides/5

in mmol/L
LDL Cholesterol = Total cholesterol – HDL Cholesterol – Triglycerides/2.2

	Increased Risk Level	Standard Risk Level	Desirable
HDL Cholesterol			
Females (mg/dl)	<45	45-65	>65
(mmol/L)	<1.16	1.16 - 1.68	>1.68
Males (mg/dl)	<35	35-55	>55
(mmol/L)	<0.90	0.90 - 1.42	>1.42
LDL Cholesterol			
(mg/dl)	>190	150 - 190	<150
(mmol/L)	>4.91	3.88 - 4.91	<3.38
Total Cholesterol			
(mg/dl)	>300	200 - 300	<200
(mmol/L)	>7.76	5.17 - 7.76	<5.17

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	32.9	101.4	32.8	100.1
SD.	0.3	0.7	0.4	1.1
CV. %	0.8	0.7	1.3	1.1

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** HDL-Cholesterol reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.996.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 1 mg/dl.

Linearity
The reaction is linear up to Cholesterol concentration of 150 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Icterus
No significant interference from bilirubin T&D up to levels of 60 mg/dl.

EXPECTED VALUES

	mg/dl	***[mmol/L]***
Female	48.6-75	1.60-1.94
Males	41.0-58.70	1.06-1.52
Children	51.8-71.90	1.34-1.86

DYNAMIC RANGE

1- 150 mg/dl

REFERENCES

1. National Cholesterol Education Program Recommendation for Measurement of High-Density Lipoprotein Cholesterol: Executive Summary. Clin Chem. 1995; 41:1427 - 1433.
2. Friedewald , W.T. et al. Clin. Chem. 1972; 18:499.
3. Lopes- Virella, M.F. et al. Clin. Chem. 1977; 23:882

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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CE